

Amendments to the Specification:

Please delete the heading beginning at page 10, line 1, beginning with “BRIEF DESCRIPTION OF THE DRAWINGS”.

Please replace paragraph [0022] with the following amended paragraph:

[0022] The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in ~~FIG. 1~~ (SEQ ID NO:1 and SEQ ID NO:3 [[]]) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of ~~FIG. 1~~ (SEQ ID NO:2 [[]]).

Please replace paragraph [0024] with the following amended paragraph:

[0024] The polynucleotide which encodes for the mature FoxA2 polypeptide of ~~FIG. 1~~ (SEQ ID NO:2[[]]) may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Please replace paragraph [00154] with the following amended paragraph:

[00154] The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence (SEQ ID NO:1 and SEQ ID NO:3) which encodes the mature polypeptide shown in FIG. 1, (SEQ ID NO:2[[])] may be identical to the coding sequence or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of FIG. 1 SEQ ID NO:2.

Please replace paragraph [00155] with the following amended paragraph:

[00155] The polynucleotide which encodes for the mature FoxA2 polypeptide of FIG. 1 (SEQ ID NO:2[[]]) may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Please replace paragraph [00162] with the following amended paragraph:

[00162] The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The

DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the two coding variant sequences shown in FIG. 1, (SEQ ID NO:1 and SEQ ID NO:3[[]]) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of FIG. 1 SEQ ID NO:2.

Please replace paragraph [00163] with the following amended paragraph:

[00163] The polynucleotide which encodes for the mature polypeptide of FIG. 2 (SEQ ID NO:1 and SEQ ID NO:3[[]]) may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Please amend replace paragraph [00236] with the following amended paragraph:

[00236] **Conditional deletion of FoxA2 in the lung.** Triple transgenic *FoxA2*loxP/loxP, *FoxA2*-rtTA-/*tg*, (*tetO*)7-Cre-/*tg* (*FoxA2*-rtTA compound mice) and *FoxA2*loxP/loxP, *CCSP*-rtTA-/*tg*, (*tetO*)7-Cre-/*tg* (*CCSP*-rtTA compound mice) mice are produced in which *FoxA2* is selectively deleted in subsets of respiratory epithelial cells in the developing lung. In these mice, the rtTA (reverse tetracycline responsive transactivator) is expressed in lung epithelial cells under control of *FoxA2* or *CCSP* promoter elements.

In the presence of doxycycline, rtTA binds to the (tetO)7CMV promoter, activating expression of Cre-recombinase, deleting exon 3 of the *FoxA2* gene (Fig. 1A). When *FoxA2*-rtTA compound and CCSP-rtTA compound mice are maintained on doxycycline from E0, pups are born at the expected Mendelian frequency. At birth, body and lung weights are not different in triple transgenic mice and their controls.

Please replace paragraph [00237] with the following amended paragraph:

[00237] **Immunohistochemistry demonstrates deletion of *FoxA2*.** To assess the efficiency of Cre-mediated gene deletion in *FoxA2*-rtTA compound and CCSP-rtTA compound mice, dams are maintained on doxycycline from E0. *FoxA2* staining is assessed at postnatal day 16 (PN16). In wild type mice, *FoxA2* is detected in epithelial cells of both conducting airways and in alveolar epithelial type II cells, consistent with previous studies (Zhou *et al.*, 1996). *FoxA2* staining is absent in most epithelial cells of peripheral conducting airways and alveoli in *FoxA2*-rtTA compound mice (Fig. 1B). Under control of CCSP-rtTA, *FoxA2* is deleted primarily in conducting airways and in restricted subsets of peripheral respiratory epithelial cells. The extent of deletion of *FoxA2* is variable in both *FoxA2* and CCSP-rtTA compound mice, ranging from complete absence of *FoxA2* staining to heterogeneous persistence of staining. *FoxA2* deletion is assessed by RNAase protection analysis (Fig. 1C), demonstrating the variable, but marked decrease in *FoxA2* mRNA in the lungs of *FoxA2* Δ/Δ mice.

Please replace paragraph [00238] with the following amended paragraph:

[00238] **Effects of *FoxA2* deletion on lung morphogenesis.** When *FoxA2*-rtTA compound mice are maintained on doxycycline from E0, approximately 50% of the pups died between PN1 and PN30 (n=24 litters). At E16.5-18.5, lung morphology is not perturbed in *FoxA2* Δ/Δ pups (Fig. 2A,B). However, by PN3, fewer peripheral lung saccules and decreased alveolar septation are observed, indicating an abnormality in postnatal alveolarization (Fig. 2C,D). Airspace enlargement, focal neutrophilic infiltrations and goblet cell hyperplasia are observed at PN16 and thereafter (Fig. 2E,F). Morphometric analysis of fractional airspace and fractional respiratory parenchyma supported the histologic assessment of the alveolar abnormalities in *FoxA2* but not CCSP-rtTA deleted mice (Fig. 3). Increased numbers of neutrophils and macrophages are observed in bronchoalveolar lavage fluid of one month old mice after deletion of *FoxA2* (supplemental Fig. 1). Differential cell counts showed a significant increase in neutrophils ($10 \pm 4.2\%$) compared to littermate controls ($(0.25 \pm 0.5\%)$, mean \pm s.e.m. by ANOVA, $P<0.05$). Some neutrophils stained for Ly-6 and the alveolar macrophages are generally MAC-3 positive (data not shown). Repeated bacterial cultures of the lung indicated no pulmonary infection. Likewise, sentinel mice did not indicate bacteria or viral pathogens in the colony. No bacteria are found on lung sections or are observed on cytopspins of BALF (data not shown).

Please replace paragraph [00239] with the following amended paragraph:

[00239] Goblet cell hyperplasia after deletion of *FoxA2*. Goblet cell hyperplasia is observed in bronchi and bronchioles after deletion of *FoxA2* in both *FoxA2-rtTA* and *CCSP-rtTA* compound mice (Fig. 1B, *inserts*). In the control mouse lung, goblet cells indicated by Alcian blue or MUC5A/C staining are rarely observed. In contrast, staining for acidic and neutral mucins is observed in conducting airways of *FoxA2Δ/Δ* mice as assessed by Alcian blue and periodic acid Schiff staining respectively (Fig. 4C-F). Likewise, extensive MUC5A/C staining is detected at the sites of goblet cell hyperplasia (Fig. 4G,H). *CCSP-rtTA* compound mice maintained on doxycycline from E0 survived postnatally. Airspace enlargement and neutrophilic infiltrates are not detected in the peripheral lung of *CCSP-rtTA* triple transgenic mice. Loss of *FoxA2* staining is less extensive in alveolar regions and more extensive in the bronchi and proximal bronchioles in the *CCSP-rtTA* compared to *FoxA2-rtTA* *FoxA2Δ/Δ* mice, consistent with the activity of the promoters. Goblet cell hyperplasia is more prominent in larger airways in the *CCSP-rtTA* deleted than the *FoxA2-rtTA*- deleted mice, consistent with sites of gene targeting in the two models (Fig. 5).

Please replace paragraph [00240] with the following amended paragraph:

[00240] Timed deletion of *FoxA2* during lung morphogenesis. In order to determine the temporal requirements for *FoxA2* during lung morphogenesis, dams or pups are

treated with doxycycline at various time periods during development. Airspace enlargement, variable neutrophilic infiltration and goblet cell hyperplasia are detected in the FoxA2-rtTA, *FoxA2Δ/Δ* mice, while no pulmonary abnormalities are observed in the littermate controls. When the FoxA2-rtTA compound mice are treated with doxycycline postnatally, from PN1 to PN16, the extent of *FoxA2* deletion is less and airspace enlargement is decreased compared to those maintained on doxycycline prenatally (Fig. 4). Airspace abnormalities are not observed in CCSP-rtTA-deleted mice (Figs. 1 and 3).

Please replace paragraph [00241] with the following amended paragraph:

[00241] Effects of *FoxA2* deletion on epithelial cell gene expression and PECAM.
Since FoxA2 influences the transcription of the *Titf1*, *Sftpb*, and *Scgb1a1* genes in vitro (Bingle and Gitlin, 1993; Bingle *et al.*, 1995; Bohinski *et al.*, 1994; Ikeda *et al.*, 1996), S1- nuclease protection assays are utilized to quantitate surfactant proteins (SP), SP-A, SP-B, FoxA2, and CCSP (Clara cell secretory protein) mRNAs at E17.5. When FoxA2-rtTA compound mice are maintained on doxycycline from E0 to E17.5-18, CCSP, SP-A, and SP-B mRNAs are significantly decreased (data not shown). Likewise, the content of SP-B in lung homogenates from surviving mice at PN16 is significantly decreased, 52.9 \pm 6% (mean \pm s.e.m., n=4) (supplemental Fig. 2). Since SP-B is critical for surfactant function, this decrease in SP-B may render the FoxA2-rtTA, *FoxA2Δ/Δ* mice more susceptible to respiratory dysfunction and death. Recent work from this laboratory demonstrated that reduction of SP-B to 20-30% of normal levels caused respiratory failure in adult mice (Melton *et al.*, 2003). Immunohistochemical staining for CCSP is

decreased in non-ciliated respiratory epithelial cells in the *FoxA2* Δ/Δ mice (supplemental Fig 3). In contrast, *FoxA2* mRNA, protein content, and immunostaining are unchanged, data not shown and (supplemental Figs. 2 and 3). At E18.5, SP-A mRNA is reduced 12.7-fold and CCSP mRNA reduced 3-fold in the *FoxA2*-rtTA *FoxA2* Δ/Δ mice compared to controls. Immunostaining for TTF-1, mature SP-B, pro-*FoxA2*, T1 α (a type I cell marker), FOXJ1 (a ciliated cell marker) and FOXA1 is not altered (Fig. 6 and supplemental Fig. 3). PECAM staining indicated normal distribution of pulmonary capillaries in the enlarged alveoli. Elastin staining is present in alveolar septa, however fewer septae are detected after *FoxA2* deletion, indicating a primary abnormality in alveolarization-septation (Fig. 6). Elastin fibers are not fragmented or shortened, indicating that alterations in alveolar size in *FoxA2* Δ/Δ mice are not associated with elastin destruction. Extent and distribution of phosphohistone-3 staining, an indicator of cell proliferation, are unchanged in the *FoxA2* deleted mice at E18.5 and PN2 (data not shown).

Please replace paragraph [00242] with the following amended paragraph:

[00242] **Pulmonary mechanics.** Since most *FoxA2*-rtTA, *FoxA2* Δ/Δ mice died or are ill by maturity, lung mechanics are assessed on 7-week-old CCSP compound mice during forced oscillatory ventilation. Airway and tissue resistance and elastance are significantly increased and compliance decreased, suggesting abnormalities in both conducting airways and alveolar regions of the *FoxA2* Δ/Δ mice (Fig. 7).

Please replace paragraph [00243] with the following amended paragraph:

[00243] Decreased FoxA2 staining in mouse models with goblet cell hyperplasia.

Increased expression of IL-4 or IL-13, deletion of FoxA2 (Glasser *et al.*, 2003; Jain-Vora *et al.*, 1997; Kuperman *et al.*, 2002; Rankin *et al.*, 1996) and allergen challenge (Tomkinson *et al.*, 2001) each cause pulmonary inflammation and goblet cell hyperplasia in vivo. We hypothesized that decreased expression of *FoxA2* may contribute to the pathogenesis of goblet cell hyperplasia. FoxA2 staining is decreased or absent in goblet cells in the airways in each of these mouse models, supporting the concept that decreased FoxA2 is associated with or required for goblet cell hyperplasia (Fig. 6). Nuclear FoxA2 staining is decreased or absent in the surface cells with characteristics of goblet cells indicated by mucin, PAS and Alcian blue staining. *FoxA2* staining is maintained in non-goblet bronchiolar epithelial cells and in basal cells that serve as precursors to goblet cells. Neither deletion of *FoxA2* nor treatment with IL-13 altered phosphohistone-3 staining in the airways undergoing goblet cell hyperplasia, indicating that goblet cells are derived by differentiation of precursor cells (basal and Clara cells) rather than from proliferation (data not shown).

Please replace paragraph [00244] with the following amended paragraph:

[00244] Effects of IL-4 on goblet cell hyperplasia and *FoxA2* are Stat-6 dependent.

Intratracheal administration of TH2 cytokines and IL-4 causes goblet cell hyperplasia in wild type but not in *Stat-6*-/- mice (Kuperman *et al.*, 1998). In control mice, FoxA2 staining is decreased or absent in goblet cells after intratracheal administration of IL-4. In

contrast, neither *FoxA2* staining nor goblet cell hyperplasia are observed in the *Stat-6*-/- mice (Fig. 9).

Please replace paragraph [00246] with the following amended paragraph:

[00246] FoxA2 inhibits transcription of the MUC5A/C gene in vitro. In order to assess whether FoxA2 directly regulated mucin expression in respiratory epithelial cells, a luciferase reporter construct containing 3.7 kb regulatory region of the mouse MUC5A/C gene is transfected with *FoxA2* into H292 cells. FoxA2 significantly inhibited the activity of the MUC5A/C-luciferase construct in a dose dependent manner (Fig. 10), suggesting that FoxA2 inhibits gene expression associated with goblet cell phenotype.

Please replace paragraph [00247] with the following amended paragraph:

[00247] Decreased FoxA2 associated with goblet cell hyperplasia in human lung disease. In order to determine the relationship between the loss of FoxA2 and goblet cell hyperplasia in humans, lung sections are obtained at autopsy or at lobectomy from 10 patients with chronic lung disease. Tissue is immunostained for FoxA2 and counterstained with Alcian blue. Five of the subjects are adults, four with cystic fibrosis and one with chronic pulmonary infection and bronchiectasis. Five subjects are infants dying in the first six months after birth with bronchopulmonary dysplasia. In all subjects, Alcian blue reactive, mucus producing cells lacked FoxA2 staining (Fig. 11A-E), while most cells lining normal airways stained for FoxA2. FoxA2 is readily detected in nuclei

of adjacent, non-goblet, Alcian blue negative epithelial cells lining both conducting and terminal airways (Fig. 11D). Loss of *FoxA2* is sufficient to cause goblet cell hyperplasia in the absence of inflammatory stimuli.

Please delete paragraph [0025] on page 10 beginning with “Figure 1”.

Please delete paragraph [0026] on page 10 beginning with “Figure 2”.

Please delete paragraph [0027] on page 11 beginning with “Figure 3”.

Please delete paragraph [0028] on page 11 beginning with “Figure 4”.

Please delete paragraph [0029] on page 11 beginning with “Figure 5”.

Please delete paragraph [0030] on page 12 beginning with “Figure 6”.

Please delete paragraph [0031] on page 12 beginning with “Figure 7”.

Please delete paragraph [0032] on page 12 beginning with “Figure 8”.

Please delete paragraph [0033] on page 12 beginning with “Figure 9”.

Please delete paragraph [0034] on page 13 beginning with “Figure 10”.

Please delete paragraph [0035] on page 13 beginning with “Figure 11”.

Please delete paragraph [0036] on page 13 beginning with “Figure 12”.

Please delete paragraph [0037] on page 14 beginning with “Figure 13”.

Please delete paragraph [0038] on page 14 beginning with “Figure 14”.

Please delete paragraph [0039] on page 14 beginning with “Figure 15”.